Journal of Surgical Oncology 63:41–45 (1996)

Correlation of DNA Ploidy and Histologic Diagnosis From Prostate Core-Needle Biopsies: Is DNA Ploidy More Sensitive Than Histology for the Diagnosis of Carcinoma in Small Specimens?

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DNA ploidy has been shown to have prognostic value in adenocarcinoma of the prostate. While occasional benign lesions of the prostate may be associated with a DNA aneuploid status, most aneuploid epithelial proliferations of the prostate are carcinomas. Because of the relationship between aneuploidy and malignancy, DNA ploidy analysis might improve detection of adenocarcinoma in small core-needle biopsy specimens. In this study, DNA ploidy analysis was performed on 186 fresh core biopsies from 32 patients who had undergone transrectal, ultrasonographically directed core-needle biopsies. Ploidy level was determined by Feulgen staining and image analysis with a CAS 200™ image analyzer (Becton Dickinson-Cellular Imaging Systems, San Jose, CA). The resultant DNA ploidy levels were compared with the initial histologic diagnosis and subsequent clinical and pathologic follow-up. Nondiploid DNA patterns correlated with a diagnosis of carcinoma on core biopsy in 11 of 16 nondiploid cases and with a final diagnosis of malignancy in 13 of 16 nondiploid cases. Two patients with biopsy proven carcinoma had DNA diploid tumor patterns. Ploidy analysis had a sensitivity of 86.6% and a specificity of 73.7% in predicting the final diagnosis of malignancy. One case interpreted as DNA tetraploid by image analysis revealed seminal vesicle tissue on both the cytologic preparations and the core biopsy. Two DNA aneuploid specimen associated with cores initially read as benign or atypical demonstrated adenocarcinoma either on review of the original core biopsy or the prostatectomy specimen. The final DNA aneuploid specimen revealed acute prostatitis in the core biopsy. DNA ploidy analysis of core biopsy specimens appears to have relatively good specificity and sensitivity for the detection of prostatic carcinoma. Sampling errors appear to be the major cause of false negative results. Inappropriate measurement of seminal vesicle tissue and acute prostatitis can result in false positive results.

KEY WORDS: DNA ploidy, prostate, adenocarcinoma, core-needle biopsy

Accepted for publication April 29, 1996.

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INTRODUCTION

Many studies using flow cytometry or image analysis have demonstrated the prognostic value of DNA ploidy determinations [1–21]. Several of these studies also have shown a close relationship between DNA ploidy and tumor volume [22,23], grade [1,22,23] and stage [12–14,23–26]. Generally, higher grades of carcinoma are associated with an aneuploid pattern [1,22]. Because tumor grade and stage are not precise predictors of prognosis, DNA ploidy has been recommended as an additional prognostic study.

The majority of prostatic nodules are currently investigated by small core biopsy and histopathologic diagnosis of carcinoma in these biopsy specimens may be difficult. A nondiploid state is known to correlate with the presence of carcinoma, although rare benign proliferation may possess an aneuploid DNA cell population. Recently, Warzynski et al. [27] reported their success with performing DNA ploidy assays on paraffin-embedded biopsy core specimens performed using flow cytometry. Herein, we report our experience using image analysis to determine DNA ploidy in a series of 186 core biopsy specimens (32 patients). We found DNA ploidy determinations to be useful for establishing the diagnosis of adenocarcinoma in small core biopsies of the prostate.

MATERIALS AND METHODS

Eighteen-gauge needle-core biopsy specimens (186) of prostate were obtained from 32 patients who had undergone transrectal ultrasound guided random and directed prostate biopsies during July 1992 through September 1992. The age of the patients ranged from 49–80 years with a mean patient age of 66 years. Serum prostate-specific antigen levels ranged from 0.2 to 107 ng/ml with a median level of 9.6 ng/ml. Touch imprints were taken from each of the fresh core biopsy specimens and labeled as to the biopsy core location.

These touch imprints were air-dried for 24 hours, then fixed in 10% neutral-buffered formalin for 30 minutes. The imprints were then rinsed in running deionized water for 5 minutes and placed in 5 N hydrochloride acid for 60 minutes. They were subsequently stained with DNA Feulgen stain, dehydrated with ethyl alcohol, cleared in xylene, and coverslipped. A calibration slide with predeposited rat tetraploid hepatocytes accompanied each staining batch (Becton Dickinson-Cellular Imaging Systems).

Prior to any knowledge of the histopathology results, these fresh touch imprints were analyzed for DNA content by the CAS 200 Image Analyzer™ utilizing the quantitative ploidy analysis software program (Becton Dickinson-Cellular Imaging Systems). Twenty control cells with a known DNA content (rat tetraploid hepatocytes, 6.7 pg) were analyzed to determine the optical density of each staining batch. Lymphocytes in the specimens were measured as an internal diploid control (1.0+/−10%). The DNA content of 100 cells based on morphologic assessment from every specimen slide was analyzed. Degenerating tumor nuclei, obviously artifactually distorted nuclei, and overlapping nuclei were rejected by the operator (BJK). A frequency histogram of cell count versus DNA content for the entire 100 cells of the specimen was generated. Results were expressed as both a mean DNA content in picograms (pg) and a DNA index (DI).

The results were reported as diploid and nondiploid. Diploid was defined as having a main peak DNA index of 0.9–1.20 and nondiploid was reported as having a main peak DNA index of >1.20. In addition, the DNA index for stroma was measured as a second diploid internal control.

Of the 32 patients, 10 underwent a subsequent prostatectomy. All surgeries occurred in the time span of <1 month to $1\frac{1}{2}$ years after the core biopsy date. Quantitation of DNA content was performed on tumor cells from either fresh touch imprints of the prostate or formalin-fixed paraffin embedded tissue sections cut at 5 μm and 7 μm using the staining protocol and CAS 200 Image Analysis System™ in the same procedure as described above.

RESULTS

The correlation of DNA ploidy with cytologic (imprint), histologic, and clinical follow-up in 32 men undergoing prostate needle biopsy is found in Table I. Thirteen of 32 (41%) patients had at least one core biopsy sample histologically positive for adenocarcinoma. Two of 32 (6%) core biopsies were interpreted as benign on initial histologic review. Malignant cells were identified cytologically during cell selection for DNA ploidy analysis in 11 of 15 cases (73%). These cases demonstrated carcinoma with histologic study of the core biopsy specimens or during follow-up. Two of 15 malignancies (13%) demonstrated a DNA diploid pattern. Six of 15 (40%) were DNA tetraploid and seven of 15 (47%) were DNA aneuploid. Image analysis demonstrated a nondiploid cell population in 11 of 13 (85%) imprints of core biopsies containing histologically proven adenocarcinoma and in 86.6% (13 of 15) of cases with adenocarcinoma on follow-up. Two cases contained atypical glands in the core biopsy, one was DNA diploid, the other DNA aneuploid. The DNA aneuploid case with atypical glands revealed adenocarcinoma on follow-up. The second case of atypical glands associated with a DNA diploid pattern demonstrated a benign clinical outcome.

Nondiploid cells were identified in four (2 tetraploid, 2 aneuploid) of 16 (25%) histopathologically benign core biopsies. On follow-up, one of these revealed adenocarcinoma, one acute prostatitis, and a third contained seminal vesicle tissue within the core and in the imprint. The fourth and final discrepancy was unexplained.

TABLE I. Correlation of DNA Ploidy With Cytologic (imprint), Histologic, and Clinical Follow-Up in 32 Men Undergoing Prostate Needle Biopsy

DNA ploidy	Cytologic diagnosis	Histologic diagnosis	Follow-up
Diploid	Benign	Atypical glands	Benign
Diploid	Benign	Carcinoma	Carcinoma
Diploid	Benign	BPH[a]	Benign
Tetraploid	Carcinoma	Carcinoma	Carcinoma
Diploid	Benign	BPH	Benign
Diploid	Benign	BPH	Benign
Aneuploid	Carcinoma	Carcinoma	Carcinoma
Diploid	Benign	BPH	Benign
Diploid	Benign	BPH	Benign
Diploid	Benign	Carcinoma	Carcinoma
Aneuploid	Atypical cells	BPH	Carcinoma on review
Aneuploid	Carcinoma	Carcinoma	Carcinoma
Aneuploid	Carcinoma	Atypical glands	Carcinoma on review
Diploid	Benign	BPH	Benign
Diploid	Benign	Benign	Benign
Aneuploid	Atypical cells	Carcinoma	Carcinoma
Diploid	Benign	BPH	Benign
Diploid	Benign	BPH	Benign
Diploid	Benign	BPH	Benign
Tetraploid	Seminal vesicle	BPH	Benign
Diploid	Benign	BPH	Benign
Tetraploid	Carcinoma	Carcinoma	Carcinoma
Aneuploid	Carcinoma	Carcinoma	Carcinoma
Tetraploid	Carcinoma	Carcinoma	Carcinoma
Diploid	Benign	Benign	Benign
Aneuploid	Atypical cells	Benign	Benign
Tetraploid	Carcinoma	Carcinoma	Carcinoma
Diploid	Benign	Benign	Benign
Aneuploid	Carcinoma	Carcinoma	Carcinoma
Tetraploid	Benign	Benign	Acute prostatitis
Tetraploid	Carcinoma	Carcinoma	Carcinoma
Aneuploid	Carcinoma	Carcinoma	Carcinoma

[a]Benign prostatic hyperplasia.

DISCUSSION

The precise utility of DNA ploidy determinations in prostatic carcinoma remains controversial despite several studies demonstrating an independent prognostic value for DNA ploidy [1–8]. Although demonstrating a relationship between DNA ploidy and outcome, others have been unable to establish an independent prognostic value of DNA ploidy analysis [9–15]. The majority of studies have used flow cytometry [2–8,12–15], but several investigators have documented the utility of DNA ploidy analyzed as a prognostic indicator when utilizing image analysis [9–11,16,17,19–21]. Both flow cytometric and image analysis based studies have reached similar conclusions with the majority of authors finding DNA ploidy to be a significant predicator of clinical outcome. The two techniques are somewhat complementary with flow cytometry apparently superior for the identification of modal value or DNA index of nondiploid populations, but image cytometry being more sensitive for the documentation of tetraploid stem lines and of rare aneuploid peaks. Image analysis also has the advantage of being able to use smaller samples and identifying the specific populations of cells under analysis [22].

Most studies have shown a correlation between DNA ploidy and tumor grade [22], but exceptions exist [7,15,23]. Whereas DNA ploidy and tumor grade appear to be closely linked, ploidy appears to maintain an independent prognostic value in some studies [2]. DNA ploidy assessment by cytometric techniques represents a less subjective methodology for prediction of outcome than grading.

DNA ploidy correlates closely with tumor volume [22]. Tumor volume is known to be an important predictor of prognosis. Many studies have shown a good correlation between DNA ploidy and size of neoplasm and stage of disease [2,12–24,26]. Thus DNA ploidy may not only be prognostic of overall patient survival but may predict tumor volume and stage.

Because selection of preoperative therapy (radiation, hormone, chemotherapy) or the selection of operative

vs. nonoperative management may become increasingly important issues in the treatment of patients with prostatic carcinoma, prediction of tumor volume, pathologic stage, and overall prognosis from small needle biopsy specimens will become more important. DNA ploidy analysis appears to offer a reliable method for such prediction. Clearly, issues of tumor heterogeneity and methodologic sources of error need to be addressed [22] before the widespread use of these techniques can occur.

Our study demonstrated the practicality of performing DNA ploidy analysis by image analysis of small biopsy cores of prostate. Others have had a similar experience using flow cytometry [27] and image analysis [28]. In the present study of 186 core biopsies from 32 patients, DNA ploidy results were obtainable in all cases. The ploidy analysis demonstrated a sensitivity of 86.6% and a specificity of 74% for the detection of histologically proven carcinoma. The presence of diploid malignancies and sampling artifacts accounts for the reduced sensitivity of the technique in the prediction of malignancy. A significant percentage of prostatic adenocarcinomas are DNA diploid. Low grade carcinomas are more likely to be diploid than high grade carcinomas [15,16]. This represents a significant limitation for the technique of DNA ploidy analysis in the prediction of malignancy. The low grade neoplasms are the most likely to be associated with a diploid state. Nonetheless, we were able to identify two carcinomas not recognized histologically in the core biopsies and confirm carcinoma in an additional two cases where the histologic diagnosis had been difficult to establish.

The relatively low specificity of DNA ploidy analysis appeared to be due to multiple factors. In one case, a tetraploid peak was obtained from imprint preparations contaminated with seminal vesicle tissue. Seminal vesicle tissue may contain nondiploid cell populations. In a second case, acute prostatitis was present in the core biopsy. The corresponding touch imprints contained a population of cells with an increased proliferation index along with nuclear debris, which apparently resulted in an incorrect interpretation of a nondiploid cell population. Review of the histologic and cytologic material should have removed this case from this neoplastic category despite the ploidy findings. No explanation could be found for the DNA ploidy findings in a third case. The material appeared histologically benign and follow-up demonstrated no evidence of malignancy. Rarely, DNA aneuploid cell populations have been reported in benign hyperplastic lesions of the prostate [15]. This occurence may explain the present finding as well as represent a second significant limitation to the use of DNA ploidy for the diagnosis of malignancy in prostate biopsies.

A second area where DNA ploidy can be helpful in the management of prostatic carcinoma is the use of DNA ploidy results to predict survival, response to therapy, and preoperative estimation of tumor stage and grade. In many cases, preoperative estimates of prognosis using image analysis, stage, and grade would be very useful in planning therapy and the value of operative intervention. Elderly patients with low grade and stage neoplasms might benefit from nonoperative vs. operative management, whereas patients with larger higher grade neoplasms would benefit from prostatectomy. DNA ploidy appears to be a useful indicator of survival [1], tumor grade [1,22], tumor volume [29–31], and stage [2,3,12,13,23–26]. DNA ploidy performed on prostatic specimens appears independently to predict not only overall survival, but also other important pathologic features including tumor volume and stage. These pathologic findings are useful in selecting management options. Without the aid of DNA ploidy analysis, many of these prognostic features require prostatectomy specimens. They may not be available when important management decisions need to be made especially in a preoperative setting.

There are several methodologic issues of importance when considering the use of DNA ploidy analysis in small biopsy cores. The first and perhaps most important issue is that of tumor heterogeneity. It is well known that prostate neoplasms may show a significant degree of heterogeneity, and different histopathologic growth patterns are often present within a single neoplasm [22]. Such heterogeneity may represent a significant limitation in the use of small core biopsy specimens and ploidy analysis for the prediction of survival.

Despite these limitations, DNA ploidy analysis performed on small core biopsies may represent a valuable prognostic test useful in selecting therapeutic modalities as well as being an independent validation of the histologic diagnosis. When a nondiploid cell population is found in a core biopsy specimen interpreted as histologically benign, the material should be rereviewed and additional levels examined. Further biopsies should be obtained when clinically indicated. Several precautions must be taken to avoid methodologic errors [22]. Recognition of the heterogeneity of many prostatic carcinomas and the limitations of sampling should be borne in mind when evaluating DNA ploidy results. Correlation between DNA ploidy and histologic findings must be made to exclude benign causes of nondiploid peaks including specimens containing acute prostatitis and seminal vesicle tissue. With these limitations in mind, DNA ploidy performed on core biopsy specimens appears to be useful in the diagnosis of prostatic adenocarcinoma as well as in supplying prognostically useful information.

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